Further Studies of Gastrotoxic Serum. (*Progress Report.*)

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[PLATE 7.]

In two (1 and 2) previous communications to the Royal Society I was able to demonstrate the production of a gastrotoxic serum by the injection of the mucous membrane of the stomach of one animal into another. In this way, a heterogastrotoxin was formed by injecting the stomach cells of the guinea-pig into the rabbit, and an isogastrotoxin by injecting the stomach cells of the rabbit into the rabbit. I also showed that a human gastrotoxin could likewise be produced by injecting human stomach cells into another animal.

The heterogastrotoxin possesses poisonous properties for the tissues of the variety of animal whose stomach has been used for injection, but the isogastrotoxin does not. The latter, however, is toxic for the tissues of a nearly allied animal.

The gastrotoxin, on injection into an animal for which it is poisonous, produces necrotic patches in the mucous membrane of the stomach, which develop into ulcers. In the test-tube the serum also produces effects upon the tissues of the animal concerned. It was proved to be of a complex nature, and to be capable of effecting the following reactions:—

1. Hæmolysis of the red blood corpuscles.
2. Precipitation of the soluble proteids of the stomach cells, and also of other proteids of the body.
3. Agglutination of the gastric protoplasmic granules.
4. A more or less definite hyaline transformation of the intact gastric cells.

The present communication will deal with the four following points:—

1. The multiplicity of the precipitins.
2. The identity of the substances producing the precipitating and agglutinating reactions.
3. The production of immunity to the gastrotoxin.
4. The *rôle* which the gastric juice plays in the actual formation of the necrotic patches and of the subsequent gastric ulceration.

The gastrotoxic serum was in all cases obtained by injecting the washed stomach cells of the guinea-pig into the rabbit, the blood serum of the rabbit developing toxic properties for the guinea-pig's tissues.

1. *The Multiplicity of the Precipitins.*

My object was to ascertain whether the same precipitin or different precipitins were concerned in the precipitation of the various proteids.

Method.—The proteid solutions examined were albuminous extracts of the gastric, hepatic, and intestinal cells, and the blood serum of the guinea-pig. The extracts were made by grinding up the cells to a pulp, shaking up the latter with salt solution, and filtering through a Berkefeld filter. The proteid solutions were all made of such a strength that a faint cloud was formed on testing them with potassium ferrocyanide and acetic acid. It is necessary to use the solutions of the same strengths, because the precipitate is soluble in excess of the precipitable substance, and therefore if albuminous fluids of different strengths are employed, erroneous results will follow.

If equal volumes of such a proteid solution and anti-serum be mixed together and incubated for four hours, and if the precipitate which forms in the solution be removed by centrifugalisation, the resulting solution, which will consist of a two-fold dilution of anti-serum, will be found to be incapable of precipitating any further the proteid solution. In this way one can easily remove the precipitin for any particular proteid, and the solution can then be tested with regard to its power of precipitating a different proteid. In all cases, of course, a control must be prepared, so as to prove that the precipitin has been actually removed.

In this way the precipitins for each of the four proteid solutions mentioned above were removed, and the resulting serum diluted to different degrees and tested as to its capacity of precipitating the three remaining proteids, with the following results :—

Removal of the Precipitin for Stomach-cell Proteid.—A solution of gastrotoxin from which the precipitin for stomach-cell proteid has been removed is thereby rendered quite incapable of precipitating the proteids of liver cells, intestine cells, or blood serum.

Removal of the Precipitin for Liver-cell Proteid.—Removal of the precipitin for liver-cell proteid slightly weakens the power of the gastrotoxin to precipitate the proteids of stomach cells and blood serum, and it completely deprives it of the power of precipitating intestine-cell proteid.

Removal of the Precipitin for Intestine-cell Proteid.—Removal of this precipitin renders the gastrotoxin incapable of precipitating liver-cell proteid,

but only weakens its precipitating power for the proteids of stomach cells and blood serum.

Removal of the Precipitin for the Proteid of Blood Serum.—If the precipitin for the blood serum be removed, the gastrotoxin is still able to precipitate all the three remaining solutions, but not quite to the same degree as before.

It appears, therefore, that the precipitins of gastrotoxic serum are multiple and to a great extent specific, but that their actions overlap to some extent. The gastrotoxic precipitins for liver-cell and intestine-cell proteids appear to be more nearly allied than the others, if not identical. In confirmation of the above statements I may say that if the blood serum of an immunised rabbit be examined every day, it will be found that the first precipitins to appear in the serum are those for the proteids of the stomach cells and blood serum, and that the precipitins for the liver-cell and intestine-cell proteids appear at a later date during the process of immunisation.

These results are in agreement with the statement of Nuttall (3) and others to the effect that precipitins are to a large extent, but not absolutely, specific.

2. *The Identity of the Substances producing the Precipitating and Agglutinating Reactions.*

The precipitin for stomach-cell proteid was removed from a solution of gastrotoxin in the way described above and the capacity of this solution to agglutinate gastric-cell granules then tested. In order to prove that the precipitin was removed from the solution, a control was in each case prepared, in which stomach-cell proteid was added to the solution instead of gastric granules.

In this way I have been able to show that in the absence of the precipitin no agglutination of gastric granules occurs, in other words the gastrotoxin does not contain a specific agglutinin for the gastric granules. When unwashed granules are used for the performance of the agglutination test, the gastrotoxin precipitates the albuminous fluid in which the granules float and the latter are agglutinated by this precipitate. When washed granules are used, the gastrotoxin no doubt produces some change of a coagulative nature in the superficial layers of the granules and they are therefore agglutinated as they sink to the bottom.

On the other hand there is no doubt that the gastrotoxic agglutinin for red blood corpuscles is a specific substance, because if the gastrotoxic precipitins be removed as described above, the resulting solution still agglutinates red blood corpuscles in exactly the same strengths as untreated gastrotoxic serum does. The serum is, of course, heated to 55° for half an hour before

performing this test, in order to prevent hæmolysis. My results disagree with those of observers such as Deutsch (4) and others, who hold that specific agglutinins are formed for the protoplasmic granules of cells, and who make no mention of any precipitating action. Their position, according to my experiments, is untenable.

3. *The Production of Immunity to Gastrotoxin.*

The effect of repeated injection of gastrotoxic serum is to produce immunity to the gastrotoxin.

It seemed that a determination of the results obtained from the continued administration of gastrotoxin would be interesting from two points of view:—

(1) With regard to whether chronic ulceration of the stomach could be produced in this way, or whether the animal would become immune.

(2) If the animal became immune, whether it would develop protective substances in its blood against one or all of the constituents of the gastrotoxic serum. In this way light would probably be thrown upon the actual cause of the ulceration, whether it was of hæmorrhagic origin primarily, or due to a direct action upon the gastric cells.

Method.—The method which I have found to be the most satisfactory is the intraperitoneal injection of inactive (heated to 55° C.) gastrotoxic serum.

The injected animal was, of course, the guinea-pig, and the serum was derived from rabbits immunised by injection of washed guinea-pig's stomach cells.

One injection of 5 c.c. was given each week. In order to produce a satisfactory degree of immunity, 10 or 12 injections are necessary.

In this way I have immunised 10 guinea-pigs. The immunity of these animals was tested both by experiments *in vivo* and also *in vitro.*

1. *Examination* in vivo: *Active Immunity.*—Four or five injections of the serum will not protect an animal against a lethal dose (10 c.c.) of gastrotoxin, which still produces necrotic patches in the stomach.

After an animal has, however, received 10 injections (50 c.c. serum in all) a lethal dose (10 c.c.) of gastrotoxin fails to produce necrotic patches in the stomach of that animal (fig. 1, Plate 7).

Passive Immunity.—The blood serum of an actively immune animal is capable of conferring passive immunity upon another animal.

In an experiment of this kind a lethal dose (9 c.c.) of active gastrotoxic serum was mixed *in vitro* with 6 c.c. inactive protective serum and the mixture injected into a guinea-pig weighing 360 grammes. No lesion was produced in the stomach. The control animal was inoculated with 9 c.c. active gastrotoxic serum and 6 c.c. inactive normal guinea-pig's serum. This

animal showed the usual patches of necrosis in its stomach. Normal guinea-pig's serum is itself able to protect an animal to a slight extent (fig. 2).

2. *Examination* in vitro.—The examination was conducted from two points of view:—

(*a*) To find out whether the tissues of an immune animal were as susceptible to the gastrotoxin as were those of a normal animal.

(*b*) To ascertain what protective power the serum of the immune animal possessed with regard to preventing the action of gastrotoxin *in vitro*.

(*a*) *Action upon the Tissues of an Immune Animal: Blood Corpuscles.*—The gastrotoxic hæmolysin dissolves the red corpuscles of an immune animal to exactly the same degree as it does those of a normal guinea-pig.

Proteid Solutions.—The stomach proteid, intestine proteid, and even the blood serum of the immune animal are all precipitated by the gastrotoxic serum.

Stomach Cells.—The same action is seen upon the immune cells as upon the normal cells, namely, more or less clearing of the cells.

In the case of several other poisons it has been proved that the tissues of the immunised animal are still acted upon by the poison.

(*b*) *Protective Power of the Serum* in vitro.—The protective serum was, in all cases, heated to put the complement out of action.

Hæmolysin.—Hæmolysis is completely prevented by the immune serum.

The immune serum obtained from an animal after four injections prevents hæmolysis to some extent. After eight injections it has more power. After 11 injections 0·5 c.c. serum completely prevented any hæmolysis occurring in any of the tubes. (Hæmolytic strength of the serum used:—0·25 c.c. serum completely dissolved 1 c.c. 5-per-cent. suspension of guinea-pig's corpuscles.)

Probably smaller amounts than this would suffice. The experiments were only made, however, to demonstrate the presence of an anti-hæmolysin.

Precipitin.—I have not been able to show the presence of any anti-precipitin. The serum of an immune animal, on being added to the gastrotoxin in sufficient amount to dissolve any precipitate that forms, fails to prevent the precipitation of stomach-cell proteid.

Action on the Gastric Cells.—The changes in the cells are not sufficiently definite for any very accurate determinations to be made, but my impression is that the cells are as much acted upon, whether the immune serum is present or not. This point must, therefore, for the present be left an open question.

Anti-precipitins have been described, but my results fail to confirm such observations.

Anti-hæmolysins have, of course, been recognised for a long while.

4. *The Rôle which the Gastric Juice plays in the Production of Gastric Ulceration.*

I have been able to prove that the actual ulceration produced by injection of the gastrotoxic serum is brought about by the action of the gastric juice.

Although it is considered at the present day that the gastric juice plays an important part in the production of gastric ulcer, I have not been able to find any reliable experimental evidence in support of such a supposition. Of course, it is clear that if the gastric cells are killed by any means, the gastric juice will digest them, as it does any foreign proteid substance, but I refer to self-digestion of the stomach, in which the gastric juice attacks a gastric cell which shows no deviation from the normal on microscopic examination.

The whole question of self-digestion of the stomach is extremely interesting and important, but as yet very little is known about the subject.

Method.—My method is to neutralise the gastric juice with sodium bicarbonate solution before injection of the gastrotoxic serum.

In order to do this a soft rubber catheter is passed down the œsophagus of a guinea-pig into its stomach, and about 14 c.c. of a 4-per-cent. sodium bicarbonate solution introduced through a small funnel. The gastrotoxic serum is then injected into the animal's peritoneal cavity. A control pig is at the same time injected with the same amount of the same serum. I have done eight such experiments, and in all the cases in which the gastric juice was previously neutralised no necrosis of the stomach could be seen, but in all the control animals the usual black patches were visible in the mucous membrane of the stomach (fig. 3).

The stomach contents in the pigs which were previously treated with alkali were found to be strongly alkaline, whilst the contents of those not so treated were strongly acid. I am not yet prepared to state the weakest strength of the soda solution which will prevent necrosis.

I have hitherto found no microscopic change in the stomach which precedes digestion.

These experiments conclusively prove what I had previously shown in another way, namely, that the initial lesion is not a hæmorrhage, for in that case neutralisation of the gastric juice would certainly not prevent a hæmorrhage occurring. It is also well known that precipitins cause no lesion on injection, because any precipitate formed would be dissolved by the excess of the animal's fluids present. It appears to me that a functional disturbance is set up in the gastric cells which renders them susceptible to

the action of the gastric juice. What that disturbance is and whether or not it bears any relationship to the slight hyaline change which I have described as occurring *in vitro*, I am not prepared to state. I think, however, that it is a different process, because the action is destroyed by heat, no visible change is to be seen in the cells, and an antibody is formed against it.

If a solution of hydrochloric acid (0·5, 1, or 2 per cent.) be introduced into the stomach previous to the injection of the gastrotoxic serum, the resulting necrotic lesions are more pronounced than they are in the control animals (fig. 4). A 2-per-cent. solution of hydrochloric acid alone produces no effect whatever when introduced into the stomach of a guinea-pig.

I think, therefore, that hyperacidity of the gastric juice must play some part in the formation of gastric ulcers; the essential factor, however, is some alteration in the cell itself, and probably this alteration may be brought about in a variety of ways.

Conclusions.

1. A gastrotoxic serum contains many precipitins which are more or less specific in their actions, but these actions overlap to some extent, and absolute specificity is unknown.

2. There is no specific agglutinin for the gastric granules, the agglutination being brought about by the precipitins.

3. By repeated injection of the gastrotoxic serum, immunity is established. The immune substance is present in the blood serum of the animal, its tissues being still susceptible to the poison.

It seems, therefore, impossible to establish a chronic lesion by gradual absorption of the gastrotoxin, if the immunising mechanism of the animal be intact. Any lesion which is produced occurs within a few hours, as I have shown. If a chronic lesion occurs as the result of the action of such a cytotoxin, the explanation is probably to be found in the perpetuation of an acutely produced lesion by some cause or other. Whether this cause be secondary bacterial infection or hyperacidity of the gastric juice remains to be proved.

4. The actual necrosis and ulceration of the stomach is produced by the gastric juice acting upon a cell which is functionally damaged. Hyperacidity of the gastric juice increases the tendency to such ulceration.

REFERENCES.

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3. Nuttall, "Blood Immunity and Relationship" (text-book).
4. Deutsch, "Sur le sérum antihépatique." XIII^{e} Cong. Internat. de Méd., Sect. de Bact. et Parasitol., 1900, Paris, 'Compt. Rend.,' pp. 55—56.

DESCRIPTION OF PLATE.

FIG. 1.—Active Immunity to Gastrotoxic Serum.

Upper stomach: This is the stomach of the control animal, which was injected with 10 c.c. gastrotoxic serum. It shows patches of necrosis of the mucous membrane.

Lower stomach: This guinea-pig had been immunised with 10 injections of gastrotoxic serum. A lethal dose (10 c.c.) of gastrotoxic serum was injected. No lesion has resulted.

FIG. 2.—Passive Immunity.

Upper stomach: This animal received a mixture of 9 c.c. gastrotoxic serum and 6 c.c. protective serum. No lesion is to be seen.

Lower stomach: This animal received a mixture of 9 c.c. gastrotoxic serum and 6 c.c. normal serum. Necrotic patches are to be seen in the stomach.

FIG. 3.—Neutralisation of the Gastric Juice by Sodium Bicarbonate.

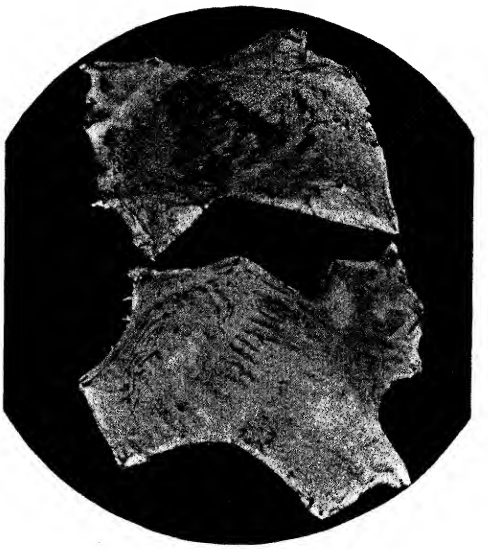
Upper stomach: This is the stomach of the control animal, which was injected with 10 c.c. gastrotoxic serum. It shows necrosis of the mucous membrane.

Lower stomach: 14 c.c. sodium bicarbonate solution (4 per cent.) were introduced into this stomach, and the animal was then injected with 10 c.c. gastrotoxic serum. No lesion has resulted.

FIG. 4.—Hyperacidity of the Gastric Juice.

Upper stomach: This is the stomach of the control animal, which was injected with 10 c.c. gastrotoxic serum. It shows some necrosis of the mucous membrane.

Lower stomach: 27 c.c. hydrochloric acid solution (1 per cent.) were introduced into this stomach, and the animal was then injected with 10 c.c. gastrotoxic serum. Extensive necrosis of the mucous membrane has resulted, and it is much more marked than in the control animal.



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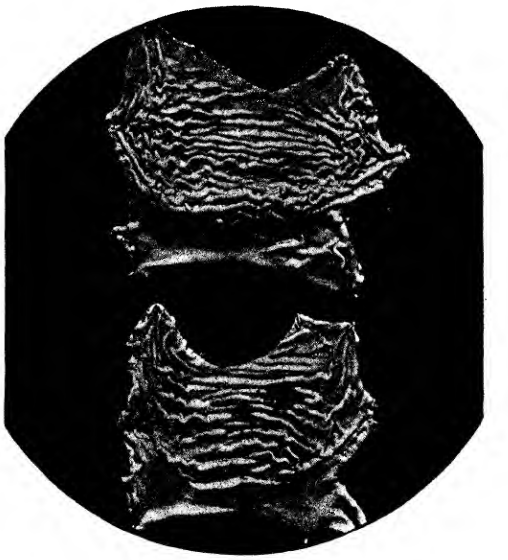

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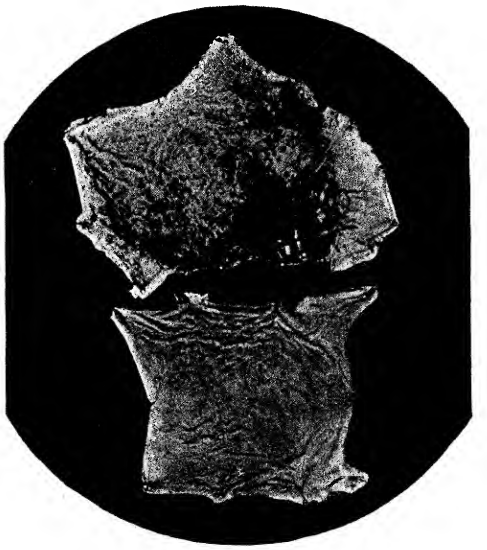

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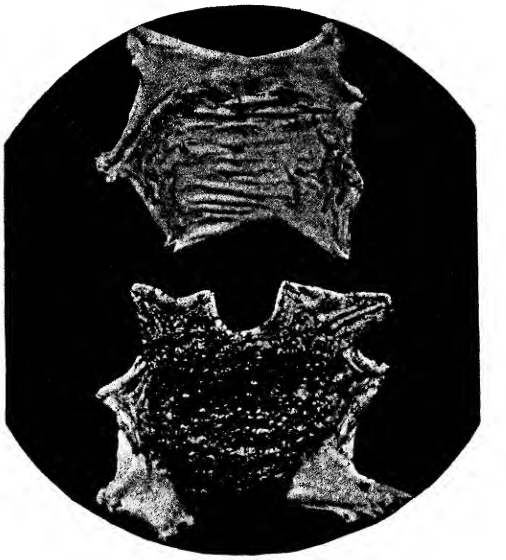

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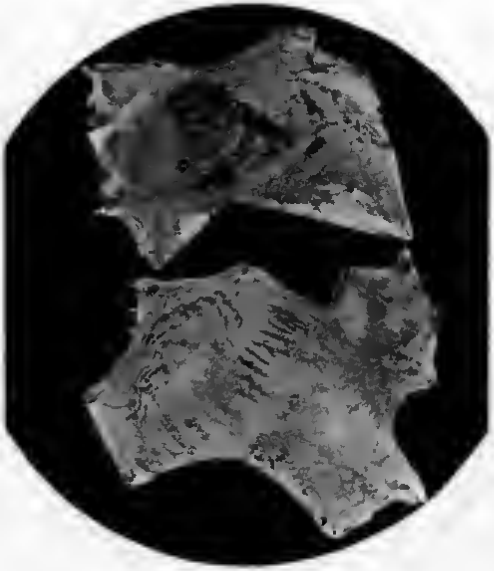

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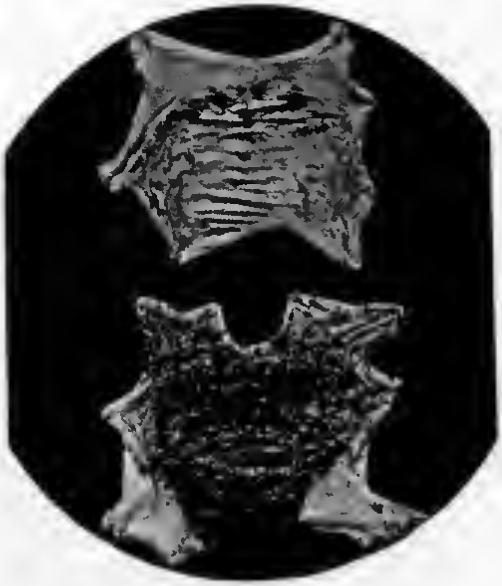

FIG. 4.